IRF4 in multiple myeloma—biology, disease and therapeutic target

Article (Accepted Version)

IRF4 in Multiple Myeloma – biology, disease and therapeutic target

Alessandro Agnarelli[1], Tim Chevassut[2] and Erika J Mancini[1*]

[1]School of Life Sciences, Biochemistry Department, University of Sussex, Falmer, Brighton, BN1 9QG, United Kingdom; [2]Brighton and Sussex Medical School, University of Sussex, Brighton, UK

[*]Corresponding author: Erika Mancini email: erika.mancini@sussex.ac.uk

Abstract:

Multiple Myeloma (MM) is an incurable hematologic malignancy characterized by abnormal proliferation of plasma cells. Interferon Regulatory Factor 4 (IRF4), a member of the interferon regulatory family of transcription factors, is central to the genesis of MM. IRF4 is highly expressed in B cells and plasma cells where it plays essential roles in controlling B cell to plasma cell differentiation and immunoglobulin class switching. Overexpression of IRF4 is found in MM patients' derived cells, often as a result of activating mutations or translocations, where it is required for their survival. In this review, we first describe the roles of IRF4 in B cells and plasma cells and then analyse the subversion of the IRF4 transcriptional network in MM. Moreover, we discuss current therapies for MM as well as direct targeting of IRF4 as a potential new therapeutic strategy.

KEYWORDS: Multiple Myeloma; IRF4; transcription regulation; Treatment; drug discovery;

1. Introduction

Multiple Myeloma (MM) is an aggressive and incurable bone marrow cancer characterized by the clonal proliferation of malignant plasma cells (PCs), the presence of monoclonal antibodies in the blood or urine and organ dysfunction [1]. MM represents approximately 2% of all cancers and about 10% of all hematologic malignancies [2] with a rising incidence estimated to be 6-10 cases per 100,000 persons per year. In the UK alone 5540 people were diagnosed and 3079 deaths were reported in 2016 by. The median age of patients at the time of diagnosis is about 65 years [2]. MM is considered a multistep disease since almost all patients with MM are characterized by an asymptomatic pre-malignant stage termed monoclonal gammopathy of undetermined significance (MGUS) and some patients by an intermediate asymptomatic but more advanced pre-malignant stage called smouldering multiple myeloma (SMM) [2] [3]. Both MGUS and SMM are characterized by the presence of the same chromosomal abnormalities of MM without clinical manifestations [3]. Moreover, MGUS evolves in MM in 1% of cases per year, while SMM progresses to MM in 10% of cases per year for the first 5 years following diagnosis [2].

Novel immunomodulatory drugs such as thalidomide and lenalidomide and proteasome inhibitors such as bortezomib, together with autologous stem cell transplantation when possible, have dramatically improved the median overall survival of patients. Relapse however will occur in all myeloma patients and the median overall survival following resistance to both bortezomib and thalidomide or lenalidomide is 9 months [4]. There is a clear need for new treatment approaches that would be able to overcome a dismal course of the disease in these patients.

Interferon Regulatory Factor 4 (IRF4) is a transcription factor belonging to the interferon regulatory factor (IRF) family. IRFs are transcription factors playing a critical role in the regulation of immune responses, immune cell development, cell growth regulation and metabolism [5]. IRF4 is a critical regulator of the immune system and it is essential for PC differentiation [6] [7]. IRF4 has also emerged as the master regulator of an aberrant and malignancy-specific gene expression

programme in MM, where it is found to be overexpressed often as a result of activating mutations or translocations [8]. Knockdown experiments of IRF4 have shown a dramatic decrease in the viability of MM cells [8]. Yet IRF4 has not been the direct target of therapeutic drug discovery programmes.

Here we describe the role of IRF4 during normal PC differentiation, the mechanism of IRF4-driven deregulation of transcriptional activity in MM and we discuss the value of new therapeutic avenues to treat MM, including the direct targeting of IRF4.

2. IRF4 structure and transcriptional activity

IRF4 is characterized by an N-terminal tryptophan pentad repeat DNA-binding domain (DBD) connected to a C-terminal interferon activation domain (IAD), critical in mediating protein-protein interactions via a linker domain (LKD) (Fig.1) [5]. The DBD domain resembles a winged helix-turn-helix motif with a 3-helix bundle (α1, α2, α3), a 4-stranded antiparallel beta-sheet (β1-β4) and two large loops (between β2 and α2 and α2 and α3) (Fig.1b). The third helix slots into the major groove of the 5′-GAAA-3′ subsequence and is the major determinant of sequence-specific binding through contacts made by arginine residues on the hydrophilic face with the phosphate backbone (Fig.1b). Three of the five invariant tryptophan residues contact DNA [9] [5].

Interestingly however, and unlike other IRF protein, IRF4 binds DNA with low affinity and requires further protein-protein interactions to bind DNA. IRF4 is essential for the expression of both GC B cell-specific and PC-specific genes and the low affinity for DNA is thought to be central to this role. Depending on its protein levels, IRF4 binds DNA as a heterodimer or a homodimer to different motifs, each motif uniquely activating the expression of genes related to GC B-cell or PC differentiation. At low protein levels, IRF4 binds as a heterodimer either the AP-1-IRF composite elements AICEs (GAAATGAGTCA or GAAANNNNTGAGTCA) with AP-1 family such as Batf

(Fig.2a,d) or the Ets-IRF composite elements EICEs (GGAANN(N)GAAA) with PU.1 (Fig.2b,e) [10] [11]. During the differentiation of B cell into PCs protein levels increase and IRF4 binds as a homodimer to the interferon sequence response elements ISREs (GAAANNGAAA) [12] (Fig.2c, f).

The low DNA binding affinity of IRF4 has been attributed to the inhibitory activity of the last 30 residues of the IAD domain [13] [10] (Fig.1a). It has been postulated that this auto-inhibitory region (AR) prevents the DBD from binding to DNA, whilst DBD interactions with transcription factor partners would release AR inhibition [10]. This hypothesis however does not explain how release of the inhibition would occur when IRF4 binds to ISRE sequences as a homodimer. Recent structural studies have shown that the AR region is a flexible unstructured peptide that does not dock into the IAD helical bundle, as seen in IRF3 [5] [14] [15]. Furthermore, the diversity in sequence homology and length of the IRF4 AR region, suggest that alternative mechanisms could induce IRF4 dimerization on DNA. SAXS studies of full length IRF4 suggests that the linker region (LKD) connecting the DBD and IAD domains most likely adopts a folded conformation able to interact with the domains located at either end of the molecule and that it may therefore play a role in the regulation of IRF4 activity [5] (Fig.1b).

3. IRF4 role in transcriptional circuitry of GC B cells and plasma cells

IRF4 is the master regulator of two mutually antagonistic programs of B and PC cells gene expression [12]. B cells play a fundamental role in the humoral immune response. During antigen-dependent activation, B cells can rearrange the constant region of the IgH region yielding antibodies with different effector functions by a process called class-switch recombination (CSR). Moreover, after antigen-dependent activation, mature B cells undergo to somatic hypermutation (SHM), a process that alters the variable regions of the immunoglobulin in order to select B cells

producing high affinity antibodies. SHM leads to the affinity maturation of B cells in germinal centres (GCs) that are transient structures within secondary lymphoid organs where B cells are selected based on their ability to produce high-affinity antibodies [16]. GCs are characterized by two compartments: the dark zone (DZ) where B cells proliferate extensively undergoing SHM and the light zone (LZ) in which B cells are selected on the basis of their affinity for the antigen. The GCs ultimately produce memory B cells and high-affinity, long-lived PCs characterized by high level of antibody secretion [17]. Molecular alterations occurring during early and late phases of B cell development can lead to the generation of lymphoid tumours.

According to the ‘’kinetic model’’ proposed by Ochiai *et al*, [12] IRF4 regulates CSR, SHM, the generation of GC B cells and PC differentiation in a temporal and dose-dependent manner [7] [6] [12]. Specifically, in the early stages of the GC reaction low levels of IRF4 and binding to AICE and EICE motifs contributes to AID expression, which is absolutely necessary for CSR and SHM [18], as well as activation of Bcl6, a key regulator of GC formation and maintenance (Fig.3a) [12] [19]. On the other hand, during PC differentiation lRF4 levels are elevated and favour binding of IRF4 to the ISREs of direct target genes such as *Prdm1*, leading to expression of Blimp1. The shift to ISREs binding therefore mediates activation of Blimp1, a key component of the PC differentiation transcription programme [20] [21], and repression of Bcl6, bringing the GC program to and end and promoting the differentiation into PCs (Fig 3b,c). IRF4 levels appear to define cell fate decisions by coordinating binding partner- and DNA-binding activity.

IRF4 is absolutely required for GC formation. Studies looking at the effect in mice of B-cell specific knockdown of IRF4, show a failure in GC formation caused by insufficient induction of Bcl6 and AID [12] [7] [6]. Bcl6 is able to control a large transcriptional network by repression [22]. Bcl6, which is highly expressed in GC B cells, facilitates their rapid proliferation in the dark zone through repression of cell cycle controlling genes, such as p53 and p21, and through inhibition of the DNA damage response that facilitates tolerance for high rates of SHM [23]. *In vivo* studies have shown

that transient expression of IRF4 directly activates but does not maintain Bcl6 expression in GC B cells [12], suggesting that IRF4 might play an essential role for the establishment but not for the maintenance of GC state. Evidence suggests that IRF4 might itself be down regulated by *Bcl6* in GC B cells [24].

Once the germinal centre is formed, IRF4 expression needs to be inhibited to avoid premature differentiation into PCs [12] [19], however the exact mechanism by which this occurs is unknown. *In vivo* studies showed that during germinal centre B-cell differentiation Bcl6 represses Blimp1 expression in cooperation with Bach2, a transcriptional repressor expressed during B cell differentiation (Fig.3a,b) [25]. Specifically, Bcl6 and Bach2 cooperate in regulating B cells GC transcriptional program by forming a complex and recruiting each other to their respective *PDRM1* DNA binding sites [25]. Moreover, *in vivo* studies showed that Bcl6 directly inhibits Myc expression and confirmed the absence of Myc expression in the centroblasts located in DZ of the GC (Fig.3b) [26] [27]. Myc repression in active proliferating DZ B cells may explain the reduced number of cell divisions in DZ which allows for the affinity maturation process in the DZ of the GC [26]. On the other hand Myc is expressed in LZ GC B cells to enable their re-entry into the DZ and the continuation of the GC reaction (Fig.3b) [26].

During PC differentiation, high levels of IRF4 induce Blimp1 expression and together they repress Bcl6 expression to terminate the GC program (Fig.3b) [20] [21] [28]. Once the differentiation of PCs has taken place, Blimp1 enhances IRF4 expression and represses *Myc* transcription causing an arrest in the PC cell cycle, as Myc is required for cell proliferation and growth (Fig.3c) [7] [28] [29]. In addition, the expression of the master regulator of B cell identity Pax5, regulated by IRF4 and regulating IRF4, Bach2 and AID expression during B-cell development (Fig.3a) is inhibited by Blimp1 once differentiation of PCs takes place (Fig.3c) [30] [31] [32] [33]. The Blimp1-mediated inhibition of Pax5 causes in turn expression of X-box binding protein 1 (XBP1), a transcription factor required for PC development, which induces Unfolded Protein Response (UPR) target

genes [33] (Fig.3c). In PCs, the UPR functions as a physiological pathway and it is activated during the early differentiation that precedes the high immunoglobulin expression [34].

Recently, a novel mechanism of IRF4-dependent gene repression during PC differentiation involving newly identified DNA binding motifs called ZICE, was described [35]. Zinc finger–IRF composite elements ZICE (GGGAANNNGAAA), composed of the zinc finger motif (GGGAA) and the IRF motif (GAAA), embed an ISRE motif which allows IRF4 to bind the ZICE sequence as a heterodimer with Ikaros or the ISRE sequence as a homodimer. Surprisingly, and despite the high levels of IRF4 during PC differentiation, IRF4 is more efficiently recruited to ZICE motifs in the presence of Ikaros. Crucially, the ZICEs were identified among a subset of IRF4 target genes whose expression is reduced upon PC differentiation, including *Ebf1* a positive regulator of B-cell activation and GC reactions [35]. This report expands the number of transcription factors that partner with IRF4 to orchestrate GC B-cell and PC differentiation and raises the question of how the delicate balance of transcription factors is accurately sustained.

4. IRF4 and Multiple Myeloma

4.1 IRF4 transcriptional network in Multiple Myeloma

IRF4 plays a central role in the pathogenesis of MM [8]. Chromosomal translocation t(6;14)x(p25;q32), which juxtaposes the immunoglobulin heavy-chain (IgH) locus to IRF4, is recurrently found in about ~21% of MM cases [36] [37]. Sequencing of paired tumour/normal samples from 203 MM patients found four IRF4 missense mutations, with three of the mutations being identical (K123R) establishing K123R as a recurrent, “hot spot” mutation in *IRF4* [38] [39]. IRF4 is highly expressed in MM patients when compared to healthy PCs [40]. Moreover, IRF4 is an important prognostic marker for MM with longer survival in patients with low IRF4 expression [40]. MM tumours are however known to be highly heterogeneous and IRF4 is overexpressed

without genetic alterations in many MM cases [8]. In the context of MM, IRF4 is known to up-regulate over 100 genes that are quiescent in healthy PCs. Most of these genes, among them *Stag2*, *CDK6*, and *Myc* are associated with cellular growth and survival [8]. A study utilizing small hairpin RNAs (shRNAs) showed that *IRF4* silencing results in loss of cell viability in 10 different MM cell line models (representing different MM subtypes most of them lacking IRF4 genetic abnormalities) suggesting that MM cells depend on the ability of IRF4 to sustain an aberrant gene transcription programme [8]. This dependency has led to proposal of the "non-oncogenic addiction" of MM cells to IRF4 where the aberrant functions of normal genes, which themselves are not classical oncogenes, is required for cancer cells survival [8] [41].

ChIP-chip analysis showed that the regulatory network that IRF4 controls in MM includes genes involved in many cellular process like cell cycle regulation, membrane biogenesis, cell death regulation, PC function [8]. This regulatory network does not reflect the genetic program of normal PCs and instead more closely resembles that of antigen stimulated mature B cells [8]. Since MM is characterized by many epigenetic alterations [42], this landscape could allow IRF4 access to loci that are usually not accessible in normal PCs [8].

A direct IRF4 target of particular interest in MM is KLF2, a transcription factor of the Krüppel zinc-finger family, a negative regulator of pre-B cell clonal expansion and B cell activation [43] (Fig.3d). Ohguchi *et al.* showed that knockdown of KLF2 caused apoptosis of MM cell lines indicating that KLF2, like IRF4, is essential for MM cells [44] [8]. Interestingly, later studies demonstrated that KLF2 and IRF4 are activated by, and in turn activate, KDM3A a member of the Jumonji C-domain-containing histone demethylases, which catalyses the removal of H3K9 mono- and dimethylation (H3K9me1 and H3K9me2) [45]. The KDM3A–KLF2–IRF4 auto positive feedback loop was shown to be important for MM cell survival and homing to the bone marrow [44]. As KDM3A regulates KLF2 and IRF4 expression through its H3K9 demethylation activity, targeting the enzymatic activity of KDM3A could therefore open an interesting therapeutic window [44].

Another direct target of IRF4 in MM is Myc (Fig.3d) [8]. Unexpectedly, IRF4 itself is also a direct target of Myc transactivation, generating an auto regulatory circuit in MM (fig.4). [8]. Moreover, ChiP assay experiments showed that IRF4 and Myc regulate each other in MM cell lines, creating a positive regulatory loop resulting in an aberrant proliferation of MM cells [8] (Fig.3d). Myc expression in MM cells is unusual since normal PCs do not express Myc due to repression by Blimp1 [29] [46] (Fig.3c). Gyory *et al*. and Ocana *et al*. reported that MM cell lines overexpressed an alternative isoform of Blimp1, called Blimp1β, when compared to normal PCs [47] [48]. Blimp1β lacks the first 101 amino-terminal residues [47] and has a disrupted PR domain, a domain with similarities to SET domains found in Histone methyltransferases [47] [49]. In addition, Blimp1β is characterized by a diminished capacity to repress target genes, like Myc [47]. The expression of the truncated protein Blimp1β could explain the inability of Blimp1 to silence Myc in MM cells.

Similarly, Bcl6 that does not normally express in healthy PCs because of inhibition by Blimp, is instead up regulated in MM cells in the bone marrow microenvironment [20] [50] (Fig.3d). Bcl6 over expression in MM cells, which is modulated at least in part via Janus kinase/STAT3 and canonical nuclear factor-κB pathways, can attenuate the DNA Damage Response, conferring a selective advantage to MM cells growth [50] [51].

4.2 Targeting the IRF4 Transcriptional Network in Multiple Myeloma

Multiple myeloma (MM) is a clonal PC malignancy characterized by the growth of tumour cells in the bone marrow and an aggressive clinical course [52]. During the past decade, the advent of proteasome inhibitors (like bortezomib) and immunomodulatory agents (like lenalidomide) has improved the treatment of MM [53], however it remains an incurable disease. Almost all patients with MM who survive initial treatment will eventually relapse and require further therapy [53]. Given that MM cells depend on IRF4 for survival, blocking its expression or interfering with its

transcriptional network might be attractive and broadly applicable therapeutic options for the many subtypes of multiple myeloma [8] (Fig.4).

One way to target IRF4 is through its upstream epigenetic regulators. Conery *et al*. reported that inhibition of the transcriptional coactivators CBP/EP300 via its bromodomain selectively abrogates the viability of multiple myeloma cell lines as a result of direct transcriptional suppression of IRF4 and of its target genes [54]. In particular, CBP/EP300 bromodomain inhibition caused down-regulation of *Myc*, suggesting that CBP/EP300 plays an important role in the regulation of the IRF4/Myc axis in MM [54]. Alzrigat *et al.* showed that the inhibition of the catalytic subunit EZH2 of the polycomb repressive complex 2 (PRC2) causes a reduction of MM cells viability and a down regulation of IRF4, Myc and Blimp1 expression via upregulation of potent tumour suppressor microRNAs miR-125a-3p and miR-320c [55].

Alternatively, direct transactional activators upstream of IRF4 could be targeted. Immunomodulatory drugs (IMiDs) like lenalidomide and thalidomide have been shown to have potent anti-tumour activities in MM resulting from IMiDs/Cereblon-mediated selective degradation of transcription factors IKZF1 (Ikaros) and IKZF3 (Aiolos), which are direct transcriptional regulators of IRF4 [56] [57] [58].

Growing experimental and clinical evidence underscore the importance of natural killer (NK) cells in the immune response against MM. Combination therapies that also enhance the activity of NK cells against MM are showing promise in treating this hematologic cancer. For example, inhibition of BET through its bromodomain causes an increase of NK cell-activating MICA ligand in MM cells resulting in enhanced NK cell-mediated cytotoxicity and an upregulation of the tumour suppressor microRNA-125b-5p (miR-125b), involved in the downregulation of IRF4 expression and of its downstream signalling [59] [60]. Incidentally, IMiD drug lenalidomide has both tumouricidal and immunomodulatory activity in MM as the Cereblon-dependent degradation of IKZF1/3 proteins also causes an increase of NK cell-activating ligands MICA and PVR [61].

Given the importance of the MM- specific auto regulatory loop between IRF4 and Myc [8], IRF4 could be down regulated by direct targeting of Myc. Previous studies show that knockdown of Myc results in a decreased viability of MM cells [8], whilst inhibition of Myc-Max heterodimerization, by the small-molecule compound 10058-F4, causes MM cell death [62]. Since BET protein BRD4 directly regulates Myc expression in MM cells [63] [64], treatment of MM cells with BET inhibitor JQ1 causes release of BRD4 from the *Myc* promoter resulting in the reduction of proliferation and viability of MM cells [63] [64]. Incidentally, BET inhibitor JQ1 also suppresses the secretion of the key survival factor IL-6 in MM cells [65].

Another strategy to target IRF4 could be to disrupt MM-specific IRF4 direct protein-protein interactions, although it is not clear if IRF4 directly interacts with other protein in the MM context. Co-occupancy of EICE composite motifs sites by PU.1 and IRF4 is important for gene regulation during B cell activation [12]. During PC differentiation however high concentrations of IRF4 promote binding to ISRE motifs, upregulating PC specific genes, like *PDRM1*, and inhibiting PU.1 (*Spib1*) [12] [20]. In the majority of MM cells studied, PU.1 has been shown to be down regulated [66], however induced overexpression of PU.1 in MM cells causes down regulation of IRF4 expression and cell death by activation of the IRF7-INFβ pathway [67]. Up regulation of PU.1 could therefore represent a promising therapeutic strategy for MM.

The MM specific IRF4 aberrant downstream transcriptional network could also represent a valid target for inhibition. Previous studies reported that knockdown of Blimp1 by short hairpin RNA causes apoptosis in MM cells [68] and that the interaction between Ailos and Blimp1 plays an important role in MM cells survival, probably through the collaborative down-regulation of pro-apoptotic genes [69]. Moreover, treatment with IMiD lenalidomide caused proteasomal degradation of Blimp1 and reduced Aiolos levels leading to apoptosis of MM cells [69].

MM cells secrete an excess of monoclonal proteins and a stringent endoplasmatic reticulum (ER) quality control is essential for these high levels of protein synthesis [70]. During ER stress,

activated IRE1α protein mediates splicing of the XBP1 mRNA and fully initiates the UPR [71]. Because of its fundamental role in ER quality control, Xbp1 is highly expressed in MM cells and is required for their growth and survival [70]. Mimura *et al.* showed that targeting the IRE1α-Xbp1 pathway by small-molecule inhibition results in a decrease of MM cell viability [70]. Moreover, IRE1α-Xbp1 pathway inhibition in MM cells causes an enhanced activity of the proteasome inhibitor (PI) drug bortezomib [70].

5. Summary and future directions

Despite the introduction of novel immunomodulatory drugs, such as thalidomide and lenalidomide and proteasome inhibitors such as bortezomib, MM remains an incurable cancer. In this review, we firstly examined the biology of IRF4 in healthy B cells and PCs and we then focused on the aberrant IRF4-transcriptional network, characteristic of MM cells. Knockdown experiments and pharmacological suppression have both shown significant decrease in the viability of MM cells [8] [54] confirming IRF4 as an attractive target for novel therapeutic strategies (Fig.4).. Various pharmacological approaches have been discussed based on the inhibition of IRF4 MM-specific upstream and downstream pathways.

Future work might focus on the direct inhibition of IRF4, as currently no IRF4-specific small molecule inhibitor is available. Concentration induced homodimerization and consequent binding to ISRE composite motifs has been shown to be a requirement for IRF4 to mediate its transcriptional activity in the context of PCs [12]. Overexpression in MM suggests that homodimerization is the prevalent IRF4 mode of action in MM cells and that targeting such dimerization could constitute a valid approach to MM subversion. This hypothesis is arguably validated by the observation that mice with only one *Irf4* allele are phenotypically normal, whilst 50% decrease of IRF4 at both the mRNA and protein levels causes MM cell death [8] [72]. An IRF4-directed therapy could therefore kill MM cells with little or negligible effect effects on normal cells making it potentially an exciting therapeutic avenue for MM patients.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of Interest

The authors declare no conflict of interest

References

1. Palumbo , A. and K. Anderson *Multiple Myeloma.* New England Journal of Medicine, 2011. **364**(11): p. 1046-1060.
2. Rajkumar, S.V., *Multiple myeloma: 2016 update on diagnosis, risk-stratification, and management.* Am J Hematol, 2016. **91**(7): p. 719-34.
3. Botta, C., et al., *A gene expression inflammatory signature specifically predicts multiple myeloma evolution and patients survival.* Blood Cancer J, 2016. **6**(12): p. e511.
4. Sonneveld, P. and A. Broijl, *Treatment of relapsed and refractory multiple myeloma.* Haematologica, 2016. **101**(4): p. 396-406.
5. Remesh, S.G., V. Santosh, and C.R. Escalante, *Structural Studies of IRF4 Reveal a Flexible Autoinhibitory Region and a Compact Linker Domain.* J Biol Chem, 2015. **290**(46): p. 27779-90.
6. Klein, U., et al., *Transcription factor IRF4 controls plasma cell differentiation and class-switch recombination.* Nat Immunol, 2006. **7**(7): p. 773-82.
7. Sciammas, R., et al., *Graded expression of interferon regulatory factor-4 coordinates isotype switching with plasma cell differentiation.* Immunity, 2006. **25**(2): p. 225-36.
8. Shaffer, A.L., et al., *IRF4 addiction in multiple myeloma.* Nature, 2008. **454**(7201): p. 226-31.
9. Escalante, C.R., et al., *Crystal structure of PU.1/IRF-4/DNA ternary complex.* Mol Cell, 2002. **10**(5): p. 1097-105.
10. Brass, A.L., A.Q. Zhu, and H. Singh, *Assembly requirements of PU.1-Pip (IRF-4) activator complexes: inhibiting function in vivo using fused dimers.* Embo j, 1999. **18**(4): p. 977-91.
11. Tussiwand, R., et al., *Compensatory dendritic cell development mediated by BATF-IRF interactions.* Nature, 2012. **490**(7421): p. 502-7.
12. Ochiai, K., et al., *Transcriptional regulation of germinal center B and plasma cell fates by dynamical control of IRF4.* Immunity, 2013. **38**(5): p. 918-29.
13. Brass, A.L., et al., *Pip, a lymphoid-restricted IRF, contains a regulatory domain that is important for autoinhibition and ternary complex formation with the Ets factor PU.1.* Genes Dev, 1996. **10**(18): p. 2335-47.
14. Qin, B.Y., et al., *Crystal structure of IRF-3 reveals mechanism of autoinhibition and virus-induced phosphoactivation.* Nat Struct Biol, 2003. **10**(11): p. 913-21.
15. Takahasi, K., et al., *X-ray crystal structure of IRF-3 and its functional implications.* Nat Struct Biol, 2003. **10**(11): p. 922-7.
16. Nutt, S.L., et al., *The generation of antibody-secreting plasma cells.* Nat Rev Immunol, 2015. **15**(3): p. 160-71.

17. Shlomchik, M.J. and F. Weisel, *Germinal center selection and the development of memory B and plasma cells.* Immunol Rev, 2012. **247**(1): p. 52-63.
18. Muramatsu, M., et al., *Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme.* Cell, 2000. **102**(5): p. 553-63.
19. Willis, S.N., et al., *Transcription factor IRF4 regulates germinal center cell formation through a B cell-intrinsic mechanism.* J Immunol, 2014. **192**(7): p. 3200-6.
20. Shaffer, A.L., et al., *Blimp-1 orchestrates plasma cell differentiation by extinguishing the mature B cell gene expression program.* Immunity, 2002. **17**(1): p. 51-62.
21. Saito, M., et al., *A signaling pathway mediating downregulation of BCL6 in germinal center B cells is blocked by BCL6 gene alterations in B cell lymphoma.* Cancer Cell, 2007. **12**(3): p. 280-92.
22. Basso, K. and R. Dalla-Favera, *Roles of BCL6 in normal and transformed germinal center B cells.* Immunol Rev, 2012. **247**(1): p. 172-83.
23. Basso, K., et al., *Integrated biochemical and computational approach identifies BCL6 direct target genes controlling multiple pathways in normal germinal center B cells.* Blood, 2010. **115**(5): p. 975-84.
24. Alinikula, J., et al., *Alternate pathways for Bcl6-mediated regulation of B cell to plasma cell differentiation.* Eur J Immunol, 2011. **41**(8): p. 2404-13.
25. Huang, C., et al., *Cooperative transcriptional repression by BCL6 and BACH2 in germinal center B-cell differentiation.* Blood, 2014. **123**(7): p. 1012-20.
26. Dominguez-Sola, D., et al., *c-MYC is required for germinal center selection and cyclic re-entry.* Nat Immunolg, 2012. **13**(11): p. 1083-1091.
27. Calado, D.P., et al., *MYC is essential for the formation and maintenance of germinal centers.* Nature immunology, 2012. **13**(11): p. 1092-1100.
28. Sciammas, R. and M.M. Davis, *Modular nature of Blimp-1 in the regulation of gene expression during B cell maturation.* J Immunol, 2004. **172**(9): p. 5427-40.
29. Lin, Y., K. Wong, and K. Calame, *Repression of c-myc transcription by Blimp-1, an inducer of terminal B cell differentiation.* Science, 1997. **276**(5312): p. 596-9.
30. Decker, T., et al., *Stepwise activation of enhancer and promoter regions of the B cell commitment gene Pax5 in early lymphopoiesis.* Immunity, 2009. **30**(4): p. 508-20.
31. Schebesta, A., et al., *Transcription factor Pax5 activates the chromatin of key genes involved in B cell signaling, adhesion, migration, and immune function.* Immunity, 2007. **27**(1): p. 49-63.
32. Gonda, H., et al., *The balance between Pax5 and Id2 activities is the key to AID gene expression.* J Exp Med, 2003. **198**(9): p. 1427-37.
33. Lin, K.I., et al., *Blimp-1-dependent repression of Pax-5 is required for differentiation of B cells to immunoglobulin M-secreting plasma cells.* Mol Cell Biol, 2002. **22**(13): p. 4771-80.
34. Gass, J.N., N.M. Gifford, and J.W. Brewer, *Activation of an unfolded protein response during differentiation of antibody-secreting B cells.* J Biol Chem, 2002. **277**(50): p. 49047-54.
35. Ochiai, K., et al., *Zinc finger-IRF composite elements bound by Ikaros/IRF4 complexes function as gene repression in plasma cell.* Blood Adv, 2018. **2**(8): p. 883-894.
36. Iida, S., et al., *Deregulation of MUM1/IRF4 by chromosomal translocation in multiple myeloma.* Nat Genet, 1997. **17**(2): p. 226-30.
37. Yoshida, S., et al., *Detection of MUM1/IRF4-IgH fusion in multiple myeloma.* Leukemia, 1999. **13**(11): p. 1812-6.
38. Chapman, M.A., et al., *Initial genome sequencing and analysis of multiple myeloma.* Nature, 2011. **471**(7339): p. 467-72.
39. Lohr, J.G., et al., *Widespread genetic heterogeneity in multiple myeloma: implications for targeted therapy.* Cancer Cell, 2014. **25**(1): p. 91-101.

40. Bai, H., et al., *Bone marrow IRF4 level in multiple myeloma: an indicator of peripheral blood Th17 and disease.* Oncotarget, 2017. **8**(49): p. 85392-85400.
41. Solimini, N.L., J. Luo, and S.J. Elledge, *Non-oncogene addiction and the stress phenotype of cancer cells.* Cell, 2007. **130**(6): p. 986-8.
42. Alzrigat, M., A.A. Parraga, and H. Jernberg-Wiklund, *Epigenetics in multiple myeloma: From mechanisms to therapy.* Semin Cancer Biol, 2017.
43. Winkelmann, R., et al., *KLF2--a negative regulator of pre-B cell clonal expansion and B cell activation.* PLoS One, 2014. **9**(5): p. e97953.
44. Ohguchi, H., et al., *The KDM3A-KLF2-IRF4 axis maintains myeloma cell survival.* Nat Commun, 2016. **7**: p. 10258.
45. Yamane, K., et al., *JHDM2A, a JmjC-containing H3K9 demethylase, facilitates transcription activation by androgen receptor.* Cell, 2006. **125**(3): p. 483-95.
46. Lin, K.I., Y. Lin, and K. Calame, *Repression of c-myc is necessary but not sufficient for terminal differentiation of B lymphocytes in vitro.* Mol Cell Biol, 2000. **20**(23): p. 8684-95.
47. Gyory, I., et al., *Identification of a functionally impaired positive regulatory domain I binding factor 1 transcription repressor in myeloma cell lines.* J Immunol, 2003. **170**(6): p. 3125-33.
48. Ocana, E., et al., *The expression of PRDI-BF1 beta isoform in multiple myeloma plasma cells.* Haematologica, 2006. **91**(11): p. 1579-80.
49. Huang, S., G. Shao, and L. Liu, *The PR domain of the Rb-binding zinc finger protein RIZ1 is a protein binding interface and is related to the SET domain functioning in chromatin-mediated gene expression.* J Biol Chem, 1998. **273**(26): p. 15933-9.
50. Hideshima, T., et al., *A proto-oncogene BCL6 is up-regulated in the bone marrow microenvironment in multiple myeloma cells.* Blood, 2010. **115**(18): p. 3772-5.
51. Tahara, K., et al., *Overexpression of B-cell lymphoma 6 alters gene expression profile in a myeloma cell line and is associated with decreased DNA damage response.* Cancer Sci, 2017. **108**(8): p. 1556-1564.
52. Hanbali, A., et al., *The Evolution of Prognostic Factors in Multiple Myeloma.* Advances in Hematology, 2017. **2017**: p. 4812637.
53. Naymagon, L. and M. Abdul-Hay, *Novel agents in the treatment of multiple myeloma: a review about the future.* J Hematol Oncol, 2016. **9**(1): p. 52.
54. Conery, A.R., et al., *Bromodomain inhibition of the transcriptional coactivators CBP/EP300 as a therapeutic strategy to target the IRF4 network in multiple myeloma.* Elife, 2016. **5**.
55. Alzrigat, M., et al., *EZH2 inhibition in multiple myeloma downregulates myeloma associated oncogenes and upregulates microRNAs with potential tumor suppressor functions.* Oncotarget, 2017. **8**(6): p. 10213-10224.
56. Lu, G., et al., *The myeloma drug lenalidomide promotes the cereblon-dependent destruction of Ikaros proteins.* Science, 2014. **343**(6168): p. 305-9.
57. Kronke, J., et al., *Lenalidomide causes selective degradation of IKZF1 and IKZF3 in multiple myeloma cells.* Science, 2014. **343**(6168): p. 301-5.
58. Zhu, Y.X., et al., *Identification of cereblon-binding proteins and relationship with response and survival after IMiDs in multiple myeloma.* Blood, 2014. **124**(4): p. 536-45.
59. Abruzzese, M.P., et al., *Inhibition of bromodomain and extra-terminal (BET) proteins increases NKG2D ligand MICA expression and sensitivity to NK cell-mediated cytotoxicity in multiple myeloma cells: role of cMYC-IRF4-miR-125b interplay.* J Hematol Oncol, 2016. **9**(1): p. 134.
60. Morelli, E., et al., *Selective targeting of IRF4 by synthetic microRNA-125b-5p mimics induces anti-multiple myeloma activity in vitro and in vivo.* Leukemia, 2015. **29**(11): p. 2173-83.
61. Fionda, C., et al., *The IMiDs targets IKZF-1/3 and IRF4 as novel negative regulators of NK cell-activating ligands expression in multiple myeloma.* Oncotarget, 2015. **6**(27): p. 23609-30.

62. Holien, T., et al., *Addiction to c-MYC in multiple myeloma.* Blood, 2012. **120**(12): p. 2450-3.
63. Mertz, J.A., et al., *Targeting MYC dependence in cancer by inhibiting BET bromodomains.* Proc Natl Acad Sci U S A, 2011. **108**(40): p. 16669-74.
64. Delmore, J.E., et al., *BET bromodomain inhibition as a therapeutic strategy to target c-Myc.* Cell, 2011. **146**(6): p. 904-17.
65. Ghurye, R.R., H.J. Stewart, and T.J. Chevassut, *Bromodomain inhibition by JQ1 suppresses lipopolysaccharide-stimulated interleukin-6 secretion in multiple myeloma cells.* Cytokine, 2015. **71**(2): p. 415-7.
66. Tatetsu, H., et al., *Down-regulation of PU.1 by methylation of distal regulatory elements and the promoter is required for myeloma cell growth.* Cancer Res, 2007. **67**(11): p. 5328-36.
67. Ueno, N., et al., *PU.1 acts as tumor suppressor for myeloma cells through direct transcriptional repression of IRF4.* Oncogene, 2017. **36**(31): p. 4481-4497.
68. Lin, F.R., et al., *Induction of apoptosis in plasma cells by B lymphocyte-induced maturation protein-1 knockdown.* Cancer Res, 2007. **67**(24): p. 11914-23.
69. Hung, K.H., et al., *Aiolos collaborates with Blimp-1 to regulate the survival of multiple myeloma cells.* Cell Death Differ, 2016. **23**(7): p. 1175-84.
70. Mimura, N., et al., *Blockade of XBP1 splicing by inhibition of IRE1alpha is a promising therapeutic option in multiple myeloma.* Blood, 2012. **119**(24): p. 5772-81.
71. Bravo, R., et al., *Endoplasmic reticulum and the unfolded protein response: dynamics and metabolic integration.* Int Rev Cell Mol Biol, 2013. **301**: p. 215-90.
72. Mittrucker, H.W., et al., *Requirement for the transcription factor LSIRF/IRF4 for mature B and T lymphocyte function.* Science, 1997. **275**(5299): p. 540-3.

Figure Legends

Figure 1: Overall structure of IRF4. (a) Schematic representation showing the domain arrangement of IRF4: DNA binding domain (DBD, red), linker domain (LKD), Interferon Activating Domain (IAD, blue), auto inhibitory region (AR). **(b)** Cartoon representation of the crystal structure of the IRF4 DBD bound to GAAA consensus motif [9] and IAD (PDB: 5BVI). The LKD domain, which is thought to be folded into a domain structure, interacts with both DBD and IAD domains [5]. The AR domain is flexible and does not interact with either IAD or DBD domains [5].

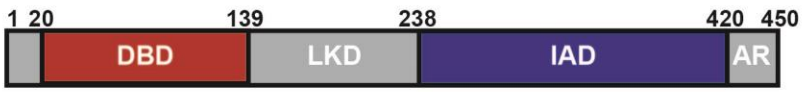
Figure 2: IRF4 cooperative DNA binding and transcription outcome. (a, b, c) Schematic representation of the different IRF4 DNA binding modes. IRF4 binds the affinity high affinity composite DNA binding motif ETS–IRF (EICE) with members of the Ets family **(a)** or AP-1-IRF (AICE) with members of the AP-1 family **(b)**. At high concentrations IRF4 binds the DNA interferon response elements (ISRE) as a homodimer **(c)**. The different outcomes of cooperation between IRF4 and other transcription factors or itself are listed. The crystal structure of the PU.1 (teal) - IRF4 (red) DNA binding domains bound on a high affinity EICE composite motif is shown in a cartoon representation **(d)**. A model of the BATF-Jun (green) - IRF4 (red) DNA binding domains complex bound on an AICE motif **(e)**, based on the known structures of the Jun-ATF2-IRF3B complex in the interferon-β enhanceosome (PDB: 1T2K) and the PU.1-IRF4 complex on the IgL λ gene enhancer [9], is shown in a cartoon representation. The model was built using AICE motifs from Bcl11b (0 bp spacing) loci. A model for the IRF4 DNA binding domain homodimer (red) bound on an ISRE motif **(f)** based on the structure of the PU.1-IRF4 complex on the IgL λ gene

enhancer [9] is shown in a cartoon representation. The model was built using an ISRE motif with 2bp spacing.

Figure 3: IRF4-transcriptional networks in activated B cells, Germinal Centers, Plasma Cells and Multiple Myeloma Cells. Schematic representation of IRF4-transcriptional networks where green and boxes denote actively expressed and repressed protein respectively **(a)** Schematic representation of the IRF4-transcriptional network in activated B cells where IRF4 is expressed at low levels (*). **(b)** Schematic representation of the IRF4-transcriptional network in GCs. IRF4 is not expressed in the Dark Zone whilst it is expressed at high levels (**) in the Light Zone. **(c)** Schematic representation of the IRF4-transcriptional network in a plasma cells where IRF4 is expressed at high level (**). **(d)** Schematic representation of the aberrant IRF4 transcriptional network in myeloma cells where IRF4 is overexpressed (**).

Figure 4: Targeting the IRF4-transcriptional network in Multiple Myeloma Cells. (a) High levels of IRF4 drive the differentiation of activated B cells into plasma cells. **(b)** Aberrant overexpression of IRF4 drives and the development of myeloma cells. **(c)** Myeloma cells are addicted to an abnormal regulatory network controlled by IRF4 and IRF4 inhibition leads cells to apoptosis.

a



b

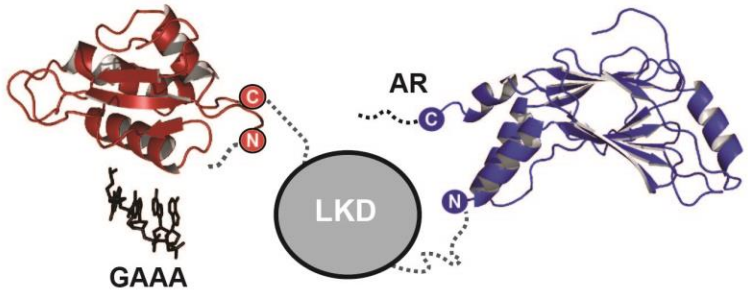


Fig.1

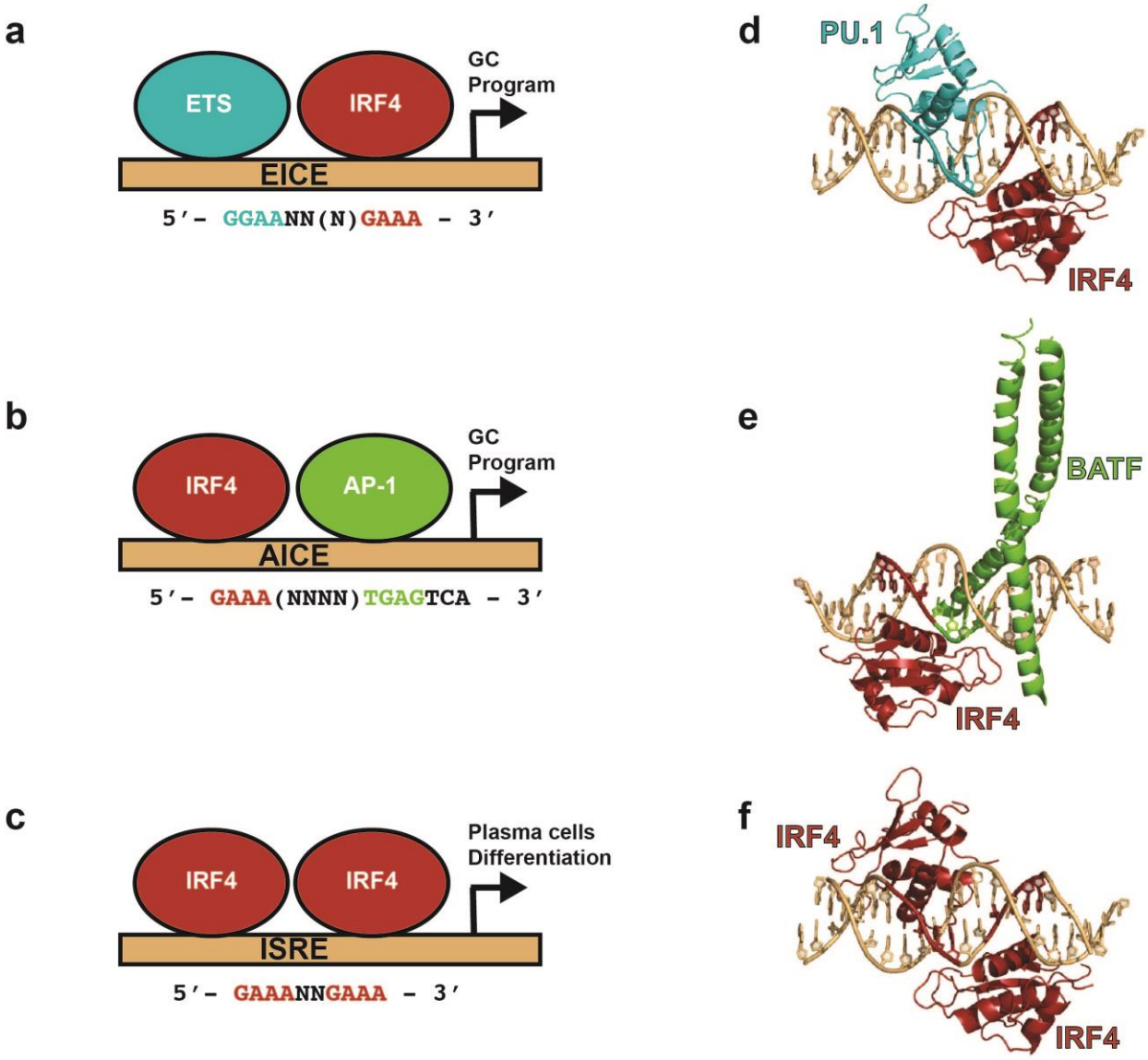

a
ETS
IRF4
GC
Program
EICE
5′– GGAANN(N)GAAA – 3′
b
IRF4
AP-1
GC
Program
AICE
5′– GAAA(NNNN)TGAGTCA – 3′
c
IRF4
IRF4
Plasma cells
Differentiation
ISRE
5′– GAAANNGAAA – 3′
d
PU.1
IRF4
e
BATF
IRF4
f
IRF4
IRF4

Fig.2

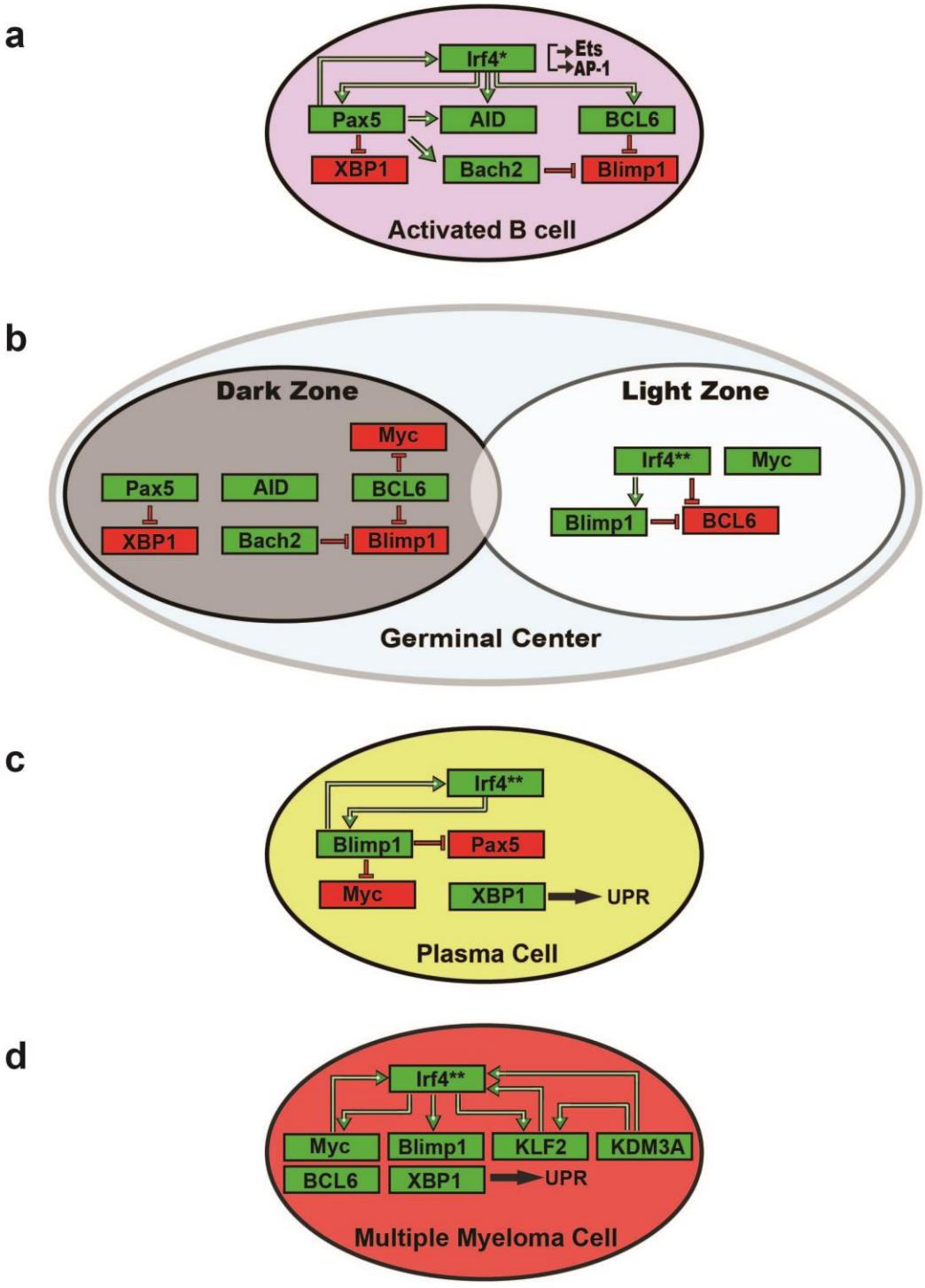

a
Irf4*
Ets
AP-1
Pax5
AID
BCL6
XBP1
Bach2
Blimp1
Activated B cell
b
Dark Zone
Light Zone
Myc
Pax5
AID
BCL6
XBP1
Bach2
Blimp1
Irf4**
Myc
Blimp1
BCL6
Germinal Center
c
Irf4**
Blimp1
Pax5
Myc
XBP1
UPR
Plasma Cell
d
Irf4**
Myc
Blimp1
KLF2
KDM3A
BCL6
XBP1
UPR
Multiple Myeloma Cell

Fig.3

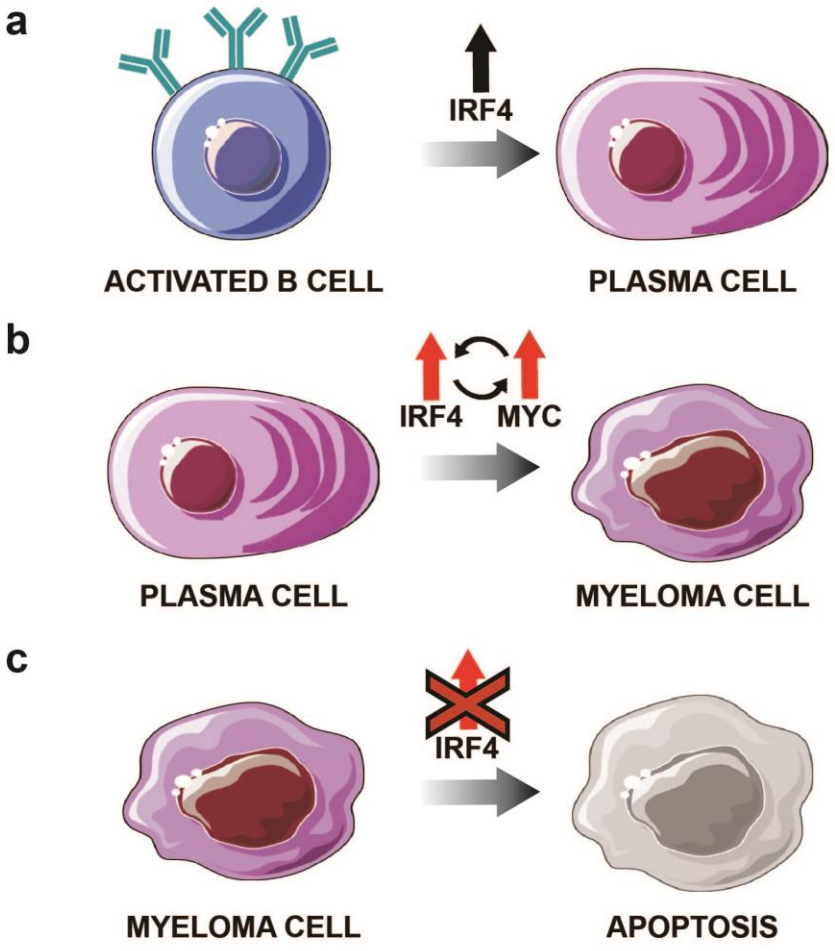

a
IRF4
ACTIVATED B CELL
PLASMA CELL
b
IRF4
MYC
PLASMA CELL
MYELOMA CELL
c
IRF4
MYELOMA CELL
APOPTOSIS

Fig.4